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Interleukin-17A enhances the production of CD147/extracellular matrix metalloproteinase inducer by monocytes from patients with psoriasis

L.M. AMEZCUA-GUERRA[1,2], M.F. ORTEGA-SPRINGALL[3], A.E. GUERRERO-PONCE[3], M.E. VEGA-MEMIJE[3], R. SPRINGALL[1]

[1]Immunology, Instituto Nacional de Cardiología Ignacio Chávez, Mexico City, Mexico
[2]Health Care, Universidad Autónoma Metropolitana – Xochimilco, Mexico City, Mexico
[3]Dermatology, Hospital General Dr. Manuel Gea González, Mexico City, Mexico

Abstract. – OBJECTIVE: CD147 is the main inducer of extracellular matrix metalloproteinases, which are critically involved in different inflammatory diseases. Our objective was to assess whether in vitro stimulation with Th1 and Th17 cytokines modulate CD147 production in monocytes from psoriasis patients.

PATIENTS AND METHODS: Serum CD147 levels were measured in 60 psoriasis patients and 60 healthy controls. Furthermore, CD14+ monocytes were cultured and stimulated with TNF, IFN-γ or IL-17A, and CD147 production was measured.

RESULTS: Serum CD147 levels were higher in psoriasis patients (median 1866, IQR 1517-2355 pg/mL vs. 1686, 1382-1947 pg/mL; *p*=0.023), allowing to distinguish between patients and controls (AUC-ROC 0.632 ± 0.0509). Baseline CD147 production was similar in monocytes from patients and controls (1298, 769-1645 pg/mL *vs.* 1290, 1048-1976 pg/ml, respectively). Stimulation with IL-17A (1638, 1426-2027 pg/mL; *p*<0.001), but no other cytokine, was associated with increased production of CD147 in monocytes from psoriatic patients. In contrast, none of the cytokines increased CD147 production in monocytes from healthy controls.

CONCLUSIONS: CD147 production by activated monocytes is a cytokine-dependent process, specifically by cytokines of the Th17 phenotype instead of those belonging to the Th1 phenotype. CD147 is a novel inflammatory mediator that could be a therapeutic target in psoriasis.

Key Words:
CD147, Interleukin-17A, Psoriasis.

Introduction

Psoriasis is an inflammatory skin disease characterized by epidermal thickening, parakeratosis, and leukocyte infiltration. These pathological features are driven mainly by monocyte-derived cytokines, which in turn are tightly regulated by the functioning of T cells[1]. Cellular microenvironment plays an important role in the onset and progression of psoriasis. An appropriate identification of molecules involved in the disease pathogenesis represents a significant challenge for the development of novel therapeutic strategies[1]. The role of T cells in psoriasis has been adequately characterized, with T helper (Th) 1, Th17 and other subsets of CD4+ cells leading a large part of the mechanisms of tissue damage. In contrast, the understanding of the pathogenic role of monocytes in psoriasis is still being elucidated[2].

CD147, also known as basigin, is an inducer of extracellular matrix metalloproteinases, whose main functions are to promote tissue remodeling, angiogenesis, and infiltration of leukocytes into the dermis. In addition to its extraordinary ability to induce matrix metalloproteinases, CD147 also mediates physiological and pathological effects through the regulation of monocarboxylate transporters and intercellular communication molecules, such as integrins and soluble growth factors[3,4]. Several studies[4,5] have found elevated levels of CD147 in the serum of patients with psoriasis. In this line of thought, we evaluated whether *in vitro* stimulation with prototypic cytokines of Th1 (tumor necrosis factor [TNF] and interferon-gamma [IFN-γ]) and Th17 (interleukin-17A [IL-17A]) phenotypes modulate the production of CD147 in monocytes from psoriasis patients.

Corresponding Author: Rashidi Springall, Ph.D; e-mail: raspringall@yahoo.com

Patients and Methods

Participants

This study was conducted in patients with a diagnosis of psoriasis from our dermatology outpatient clinic. Patients with psoriatic arthritis or any concurrent inflammatory disease and those who were under treatment with glucocorticoids or biological or synthetic antirheumatic drugs were excluded. Patients with an active infection, recent surgery or trauma, pregnancy, or neoplasia were also excluded. Clinical and laboratory data were obtained at recruitment, including an assessment of disease activity (Psoriasis Area and Severity Index, PASI). Healthy age- and sex-matched individuals were included as controls.

The study was approved by our Ethics Committee and carried out in accordance with the Declaration of Helsinki. Before inclusion, all participants signed an informed consent.

Laboratory Procedures

Four mL of fasting blood were obtained in tubes coated with clotting activator, which were centrifuged at 600 g for 15 min at 4°C, and the resulting serum was stored in aliquots at −75°C until use. In addition, 5 mL of fasting blood were obtained in Ethylenediaminetetraacetic Acid (EDTA) tubes, and mononuclear cells were obtained by density gradient centrifugation with Histopaque-1077 (Sigma-Aldrich, St. Louis, MO, USA). CD14+ monocytes were subsequently isolated using the Magnisort CD14 Positive Selection Kit (Life Technologies, Carlsbad, CA, USA). Culture conditions have already been described[6]. Briefly, a total of $5x10^5$ CD14+ monocytes per well were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) medium supplemented with 10% fetal calf serum (FCS; GIBCO, Grand Island, NY, USA) + 100 μg/mL streptomycin + 100 IU/mL penicillin + L-glutamine + 25 mM HEPES buffer + 1% non-essential amino acids. Cells were incubated for 4 hours at 37ºC and 5% CO_2. Monocytes were stimulated with recombinant human TNF (100 ng/mL), IFN-γ (10 ng/mL) or IL-17A (50 ng/mL). Supernatants were collected after 48 hours of incubation and stored in aliquots at -75°C until use. Finally, CD147 levels were measured in the supernatants, while the levels of TNF, IFN-γ, IL-17A and CD147 were measured in the serum samples. All measurements were tested by enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN, USA) following the manufacturer's instructions.

Statistical Analysis

Continuous variables are expressed as medians with interquartile range (IQR) and these were compared using the Mann-Whitney test (two independent samples) or the Kruskal-Wallis test with the Dunn's post-tests (multiple comparisons). Categorical data are expressed as percentages and these were compared using Fisher's exact test.

The area under the receiver operating characteristic curve (AUC-ROC) was calculated and the extent of the association was assessed using Spearman's Rho coefficient. A value of $p<0.05$ was set for significance. Analyses were two-tailed. Statistical calculations were performed using SPSS *v*15.0 (SPSS Inc, Chicago, IL, USA) and GraphPad Prism *v6*.07 (GraphPad Inc, La Jolla, CA, USA) software.

Results

Sixty patients with psoriasis (median age 53 years; 48% women) and 60 healthy subjects (median age 52 years; 55% women) were included. The main clinical and laboratory data are summarized in Table I. A higher body mass index, as well as a higher frequency of diabetes and hypertension, was found among psoriasis patients.

Patients had higher serum CD147 levels than controls (median 1866, IQR 1517-2355 pg/mL *vs.* 1686, 1382-1947 pg/mL; $p=0.023$). CD147 levels showed limited but evident ability to distinguish between groups, with an AUC-ROC of 0.632 ± 0.0509. However, no correlation was observed between serum CD147 levels and the extent of disease severity as assessed by the PASI (rho 0.157; $p=0.288$). Patients had higher serum levels of TNF (1403, 584-1486 pg/mL *vs.* 1058, 488-1385 pg/mL; $p=0.005$), IL-17A (1528, 1193-2291 pg/mL *vs.* 820, 581-1529 pg/mL; $p<0.001$), and IFN-γ (770, 642-968 pg/ml *vs.* 652, 557-773; $p=0.040$) than controls. No significant correlation was found between the levels of CD147 and TNF (rho -0.15), IL-17A (rho 0.10) or IFN-γ (rho -0.04) in sera from patients with psoriasis.

Figure 1 shows the level of CD147 in the supernatant of cultured monocytes. Unstimulated CD147 production was similar in monocytes from psoriasis patients and healthy individuals (1298, 769-1645 pg/mL *vs.* 1290, 1048-1976 pg/ml, respectively). In monocytes from patients, stimulation with IL-17A (1638, 1426-2027 pg/mL; $p<0.001$ compared to baseline) was associated with a significant increase in CD147 production,

Table I. Demographic and clinical features of study participants.

	Controls (n=60)	Psoriasis (n=60)	p
Age, years	52 (45-58)	53 (44-59)	0.622
Female, n (%)	33 (55)	29 (48)	0.583
Body mass index, kg/m^2	27.2 (24.6-30.0)	29.1 (26.1-33.0)	**0.028**
Type 2 diabetes, n (%)	1 (1.8)	11 (20)	**0.004**
Hypertension, n (%)	0 (0)	9 (16)	**0.002**
Current smoking, n (%)	5 (9)	13 (24)	0.071
Laboratory data			
• C-reactive protein, mg/L	1.6 (0.9-3.7)	2.5 (1.1-4.6)	0.094
• Glucose, mg/dL	101 (95-108)	102 (93-116)	0.404
• Serum creatinine, mg/dL	0.8 (0.6-0.9)	0.8 (0.7-0.9)	0.624
• Total cholesterol, mg/dL	190 (171-226)	188 (164-214)	0.410
• LDL-cholesterol, mg/dL	118 (90-135)	112 (96-135)	0.750
• Triglycerides, mg/dL	162 (115-250)	144 (104-215)	0.245
Psoriasis Area Severity Index (PASI)	–	4.3 (2.2-7.4)	
Medications			
• ACEi/ARBs, n (%)	1 (2)	10 (18)	**0.008**
• Antidiabetics, n (%)	1 (2)	11 (20)	**0.004**
• Statins, n (%)	0 (0)	4 (7)	0.118
• NSAIDs, n (%)	0 (0)	1 (2)	1.00
• Aspirin, n (%)	0 (0)	4 (7)	0.118

Definitions: LDL-cholesterol, low-density lipoprotein cholesterol; ACEi, angiotensin converting enzyme inhibitors; ARBs, angiotensin receptor blockers; NSAIDs, nonsteroidal anti-inflammatory drugs.Significant differences are in bold.

although this was not observed after TNF (1524, 1048-1857 pg/mL) or IFN-γ (1283, 761-1780 pg/mL) stimulation. Conversely, in cultured monocytes from controls, stimulation with TNF (1186, 834-1609 pg/mL), IFN-γ (1141, 769-1840 pg/mL) or IL-17A (1253, 863-1701 pg/mL) was not associated with significant changes in CD147 production. In the comparative analysis between groups, a different production of CD147 was observed after stimulation with TNF (p=0.007) and IL-17A (p=0.002), but not after stimulation with IFN-γ (p=0.857).

Discussion

This study evaluated whether exposure to prototypic Th1 and Th17 cytokines regulates CD147 production in monocytes. Here, we find that IL-17A, rather than TNF or IFN-γ, leads the pro-

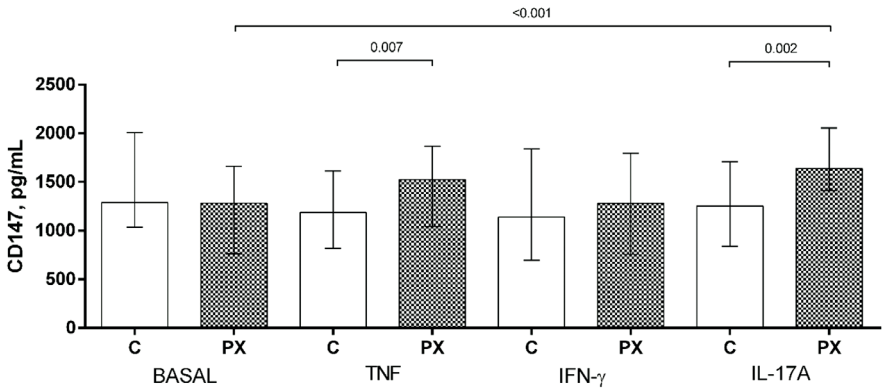

Figure 1. CD147 levels in cultured monocyte supernatant. Unstimulated (basal) production, as well as production resulting from stimulation with tumor necrosis factor (TNF), interferon-gamma (IFN-γ), and interleukin-17A (IL-17A) is represented in monocytes from both controls (C) and patients (PX).

duction of the matrix metalloproteinase inducer CD147 in activated monocytes from psoriasis patients. Notably, this response is not observed in monocytes from healthy individuals. Our results also confirm elevated circulating levels of CD147 in psoriasis patients, suggesting that this novel inflammatory mediator could be considered a potential therapeutic target in psoriasis.

The role of CD147 in psoriasis has already been highlighted. Expression of CD147 is high in neutrophils from patients with active psoriasis and its levels correlate with the extent of disease activity[4]. Furthermore, interference with CD147 expression results in a marked decrease in inflammatory cell chemotaxis[4]. Several of the mechanisms underlying the pathogenesis of psoriasis depend on numerous cytokines, with IL-17A and other cytokines belonging to the Th17 phenotype recently emerging as important humoral mediators. In fact, some IL-17A inhibitor drugs have shown high efficacy and relatively low risk in patients with psoriasis[7]. Interleukin-22 (IL-22) appears to be a critical component of the Th17 phenotype. IL-22 increases CD147 transcription both *in vitro* and *in vivo* through STAT3 activation, while CD147 knockdown strongly blocks IL-22-mediated STAT3 activation as well as IL-22-dependent chemokine production[8]. A study has described that polymorphisms in the *BSG* gene, which encodes CD147, have the ability to regulate the susceptibility to develop psoriasis. Additionally, epigenetic regulation of the *BSG* gene through microRNA also appears to modulate susceptibility to disease[9].

Finally, modulation of CD147 function may be the basis for a part of the widely recognized therapeutic effects of IL-17A inhibitors in psoriasis and, in parallel, the recent advent of therapies specifically aimed at blocking CD147 opens a new path to its potential use in psoriasis[10-11].

Conclusions

Our study showed that CD147 production from activated monocytes is a cytokine-dependent process, specifically by cytokines of the Th17 phenotype instead of those belonging to the Th1 phenotype. This positions CD147 as a novel inflammatory mediator that could be a therapeutic target for patients with psoriasis.

Conflict of Interest

The Authors declare that they have no conflict of interests

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